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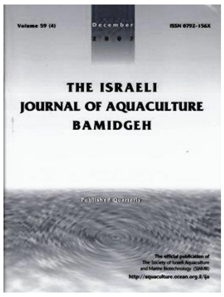

Use of Fermented Soybean Meal with Whey as a Protein Source for Feeding Juvenile Tilapia (*Oreochromis niloticus*)

Yigit Nalan Ozgur*, Demir Tulay

Suleyman Demirel University, Fisheries Faculty, Isparta, Turkey,

Keywords: tilapia; fermentation; soybean meal; growth; feed conversion ratio; whey

Abstract

In this study, the effect of fish meal substitution with fermented soybean meal in juvenile tilapia (*Oreochromis niloticus,* L.1758) diets was investigated. Six experimental diets were prepared by replacing 0 (FM, control), 10% (FSM10), 20% (FSM20), 30% (FSM30), 40% (FSM40), and 50% (FSM50) of the fish meal protein in the control diet by fermented soybean meal with whey (FSMW). The control diet contained no fermented soybean meal. The isonitrogenous and isocaloric diets were fed to juvenile tilapia (initial average weight 0.74 g) for 90 days. Each diet group was fed in triplicate to 24 fish per aquarium. At the end of the experiment, highest growth, feed efficiency, and protein digestibility were found in the group fed the FSM30 diet. Tilapia fed diets containing fermented soybean meal up to 50% fish meal exhibited similar growth, feed conversion ratio (FCR), protein efficiency ratio (PER), and protein digestibility compared with the control diet. However, feed efficiency (FE) was reduced in tilapia fed FSM50 diet. Body composition, hepatosomatic index (HIS) and viscerosomatic index (VSI) were unaffected by the dietary treatment. Results of this study showed that FSMW could replace up to 40% of fish meal protein in juvenile tilapia diets.

* Corresponding author. Tel: 090 246 211 86 46; Fax: 090 246 211 86 97; email: nalanyigit@sdu.edu.tr

Introduction

Soybean meal appears to be the most promising alternate protein source to fish meal due to reliable supply, reasonable price, and high digestibility (Anonymous 2012). However, as it contains numerous anti-nutritional factors (such as phytic acid, non-starch carbohydrates, trypsin inhibitors) use of soybean meal is limited (Reddy and Pierson, 1994; Lim et al., 2010).

Fermentation is an important process that significantly lowers anti-nutrient content (Sharma and Kapoor, 1996) and thereby improves the nutritive value of feedstuff. Previous studies showed that fermentation of soybean meal induced removal or inactivation of anti-nutritional factors (Lim et al., 2010), and improvement of nutrient digestibility (Kiers et al., 2000).

Lactic acid bacteria fermentation studies have also shown a significant reduction in phytic acid pigeon pea, (Alegbeleye, 2011) in sesame seed meal (Mukhopadhyay and Ray, 1999) and cereal (Skrede et al., 2002), in levels of non-starch carbohydrates in barley (Skrede et al., 2001; Skrede et al., 2002) and in trypsin inhibitor activity in soybean meal (Wang et al., 2007).

Whey is a by-product of the cheese industry (González-Martínez et al., 2002). Whey contains lactic acid bacteria (Mondragón-Parada et al., 2006; Shiphrah et al., 2013). In addition, whey includes some major minerals as vital nutrients (Reeves, 1982). A study regarding fermentation using whey in Japanese quails *Coturnix coturnix japonica* (Yasar and Gok 2014), reported that when fed wheat fermented with whey, weight gain and feed conversion were significantly higher.

Fermented soybean meal has also been tested in diets of several fish species, such as *Oncorhynchus mykiss* (Barnes et al., 2012), *Acanthopagrus schlegeli* (Zhou et al., 2011; Azarm and Lee 2012), *Pagrus major* (Kader et al., 2011), and *Trachinotus ovatus* (Lin et al., 2012). These studies showed that fermented soybean meal is a potential protein source in fish diets.

Tilapia is the second largest farmed finfish group in the world, after carp (FAO, 2014). Because of their rapid growth rate, good quality flesh, disease resistance, adaptability to a wide range of environmental conditions, they have become an excellent choice for aquaculture. We are unaware of any other study on the fermentation with whey in feed ingredients in fish diet. In this study we investigated the effect of replacing fish meal with fermented soybean meal on growth performance, feed efficiency, body composition, and nutrient digestibility of tilapia.

Materials and Methods

Fermentation of soybean meal with whey. All dietary ingredients were obtained locally from Abalioglu feed Company, Denizli, Turkey. Fresh whey product was obtained from Un Sut Ltd., Isparta, Turkey. Soybean meal was fermented with whey according to a process reported by Yasar and Gok (2014) and Yasar et al., (2016). Soybean meal was soaked with whey at a ratio of 1:2 and incubated at 30°C for 8 h. Then a 5 cm layer of fermented soybean meal was spread onto a clean flat table and left to dry at room temperature for 24 h. A hand rake was used to mix the mixture. When moisture content of the mixture reached 10%, the fermented soybean meal was ground in a mill and added to the experimental diets. Nutrient analyses of fermented soybean meal, unfermented soybean meal, and fish meal is shown in Table 1.

Table 1. Nutrient analyses of fermented and unfermented soybean meal, fish meal and Whey (%)

	Dry matter	*Crude protein*	*Crude fat*	*Crude fiber*	*Crude ash*
Unfermented soybean meal	91.8	44.53	1.28	4.66	5.86
Fermented soybean meal	92.39	47.33	1.28	4.51	6.17
Fish meal	92.34	67.54	8.25	-	17.33
Whey	7.10	0.60	0.34	-	1,62

Experimental diets and analysis. Six experimental diets were prepared replacing 10%, 20%, 30%, 40%, and 50% of fish meal protein in the diet with FSMW (Table 2). All experimental diets were prepared as isonitrogenous (36% crude protein) and isocaloric (16.7 MJ/kg). Chemical compositions of the diets are given in table 2.

Table 2. Formulation and chemical proximate composition of experimental diets

Ingredients (%)	*Groups* *CONTROL*	*FSM10*	*FSM20*	*FSM30*	*FSM40*	*FSM50*
Fish meal[1]	48.48	43.64	38.79	33.94	29.09	24.24
Fermented soybean meal[2]	0.00	7.11	14.22	21.33	28.44	35.56
Wheat flour[3]	32.20	32.20	32.20	32.20	32.20	32.20
Corn starch[4]	17.26	13.81	10.35	6.89	3.44	0.00
Fish oil[5]	0.46	1.60	2.75	3.91	5.05	6.19
Vitamin[6]	0.80	0.80	0.80	0.80	0.80	0.80
Mineral[7]	0.30	0.30	0.30	0.30	0.30	0.30
Cr_2O_3[8]	0.50	0.50	0.50	0.50	0.50	0.50
Methionine[9]	0.00	0.04	0.09	0.13	0.17	0.22
Chemical analysis						
Dry matter (%)	91.52	91.49	91.45	91.42	91.39	91.35
Crude protein (%)	36.62	36.68	36.74	36.79	36.85	36.90
Crude fat (%)	5.43	6.21	7.00	7.79	8.57	9.34
Crude fiber (%)	1.62	1.90	2.17	2.44	2.71	2.99
Crude ash (%)	8.34	8.24	8.14	8.03	7.93	7.83
Gross energy (MJ/g)	16.7	16.7	16.7	16.7	16.7	16.7

[1] Fish meal was provided by Abalioglu feed mill, Denizli, Turkey.
[2] Fermented soybean meal was fermented with whey in our laboratory.
[3] Wheat flour was purchased from Hediye flour, Isparta, Turkey.
[4] Corn starch was purchased from Ak Nisasta, Kırklareli, Turkey.
[5] Anchovy fish oil. Sibal Feed Mill, Sinop, Turkey
[6]Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, vitamin D3 480 000 IU, 2400 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 4000 mg Niacin, 10 000 mg Cal.D. Pantothenate, 4000 vitamin B6, 10 mg vitamin B12, 100 mg D-Biotin, 1200 mg folic acid, 40 000 mg vitamin C, 60 000 mg inositol. Kartal Chemical, Kocaeli, Turkey.
[7] Mineral premix contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinc, copper 5000 mg, cobalt 2000 mg, iodine 2750 mg, selenium 100 mg, magnesium 200 000 mg. Kartal Chemical, Kocaeli, Turkey.
[8] Aldrich-Sigma
[9] Methionine was purchased from Kartal Chemical, Kocaeli, Turkey.

In preparing the diets, fermented soybean meal and other macro ingredients were ground to small particles with a mill. Micro ingredients were first mixed and then slowly added to milled macro ingredients to ensure a homogenous mixture. Water was added to obtain a 25% moisture level. The diets were cold pelleted into 2 mm diameter size using a mincing machine. The pellets were dried at room temperature for 24 h. After drying, the diets were broken up and sieved into appropriate pellet sizes. All diets were stored at -20°C until used. The moisture, crude protein, crude fiber and ash contents of experimental diets and feces samples were determined according to standard AOAC methods (AOAC, 2002). Total lipids of all samples were determined by the chloroform-methanol extraction method (Bligh and Dyer 1959).

Fish and experimental conditions. Tilapia (*Oreochromis niloticus* L. 1758) were obtained from Fisheries Faculty, Cukurova University. The feeding trial was conducted in 18 glass aquaria (70x30x40 cm) in triplicate. At the beginning of the experiment, 25 fish (average weight 0.74±0.02 g) were randomly stocked into each aquarium. Water temperature, pH and dissolved oxygen were 27±2°C, 7.2±0.2, 6.57±0.1 mg/l throughout the experiment. Fish were fed ad libitum for 90 days. At the end of the trial, five fish from each aquarium were killed by a lethal dose of anesthesia (500 mg/L MS-222) homogenized in a blender, and stored at -20°C for subsequent protein, ash, and moisture analysis.

Digestibility study. Apparent digestibility coefficients of the different diets were measured by using 0.5% chromic oxide as a marker. Feces was collected during the feeding trial, and prepared chemical analysis as described by Lim et al., (2001). Apparent digestibility coefficients were calculated using the following equation (Cho et al., 1982):

ADC = 100 – [100×(Cr_2O_3 in diet [%]/Cr_2O_3 in feces [%])×(nutrient in feces [%]/nutrient in diet [%])].

Growth performance, feed efficiency and somatic index were calculated as follows;

Weight gain (WG) = (final body weight - initial body weight)

Specific growth rate (SGR) (% day^{-1}) = [(ln final body weight - ln initial body weight)/days] x 100

Feed conversion ratio (FCR) = feed consumed (g)/weight gain (g)

Feed intake (FI) = (total feed intake, g)/fish number

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)

Hepatosomatic index (HSI) = 100 x liver weight (g)/body weight (g)

Viscerosomatic index (VSI) = 100 x viscera weight (g)/ body weight (g)

Statistical analysis. One-way analysis of variance (ANOVA) was used to compare growth rate, feed utilization, specific growth rate, somatic indices, digestibility and body composition among treatments. All data were analyzed using SPSS computer program (SPSS, 2000). Duncan test was used to determine the differences among treatment means.

Results

Growth performance, feed efficiency, final weight, weight gain, specific growth ratio, FCR and PER of juvenile tilapia fed fermented soybean meal, is presented in Table 3. Fish fed with diets containing fermented soybean meal up to 50% of fish meal protein were similar to those of the fish fed the control diet (P>0.05). However, feed efficiency decreased in diets of tilapia fed FSM50 diet. Highest final weight (7.84 g), specific growth rate (15.74) and weight gain (7.10 g) were obtained in the FSM30 diet group while the lowest final weight (5.2 g), specific growth rate (13.11) and weight gain (4.58 g) was obtained in the FSM50 diet group.

Table 3. Growth performance, feed efficiency, protein efficiency ratio and survival ratio of tilapia fed fermented soybean meal

	Groups						
	Control	*FSM10*	*FSM20*	*FSM30*	*FSM40*	*FSM50*	*SEM*
Initial weight (g)	0.71	0.76	0.75	0.74	0.74	0.74	0.02
Final weight (g)	6.78^{ab}*	6.75^{ab}	7.43^{ab}	7.84^{a}	6.59^{ab}	5.32^{b}	0.53
WG (g)	6.07^{ab}	5.99^{ab}	6.68^{ab}	7.10^{a}	5.85^{ab}	4.58^{b}	0.45
SGR (%/day)	14.92^{ab}	14.58^{ab}	15.26^{ab}	15.74^{a}	14.38^{ab}	13.11^{b}	0.50
FCR	1.80^{ab}	1.90^{ab}	1.75^{ab}	1.65^{b}	1.79^{ab}	2.08^{a}	0.08
FI (g/fish)	9.45^{ab}	9.94^{ab}	10.40^{a}	10.47^{a}	8.75^{ab}	7.91^{b}	0.70
PER	1.16^{ab}	1.05^{ab}	1.18^{ab}	1.28^{b}	1.18^{ab}	0.89^{b}	0.09

$^{a-b*}$ Values with the same superscript in rows are not significantly different (P>0.05).

Feed intakes of groups were 9.45g in the control, 9.94g in FSM10, 10.40g in FSM20, 10.47g in FSM30, 8.75g in FSM40, 7.91g in FSM50, respectively. The FSM20 and FSM30 groups had better FCR, compared to control group. Body composition, hepatosomatic index (HSI) and viscerosomatic index (VSI) of fish fed different levels of fermented soybean meal did not show significant difference compared with the control group (P>0.05) (Table 4). Protein digestibility of fish fed with diets containing fermented soybean meal up to 50% of fish meal protein were also similar to those of the fish fed the control diet (P > 0.05). The highest protein digestibility value was found in FSM30 group (Table 4). Amount of lactic acid bacteria in diet showed increase with increase of fermented soybean meal level (P<0.05) its level in the intestine did not change with the enhancement of fermented soybean meal in the diet (P>0.05) (Table 4).

Table 4. Hepatosomatic index, viscerosomatic index, body composition, amount of lactic acid bacteria of diet and intestine, dry matter and protein digestibility of tilapia fed fermented soybean meal

	CONTROL	FSM10	FSM20	FSM30	FSM40	FSM50	SEM
Somatic index (%)							
HSI	2.21^{ab}	2.38^{ab}	1.15^{b}	1.30^{b}	2.13^{ab}	2.90^{a}	0.37
VSI	40.57	37.50	38.00	37.45	39.51	40.95	2.3
Body composition (%)							
Moisture	65.33	60.0	65.67	64.67	64.67	64.33	0.63
Crude protein	21.56	22.68	23.09	22.56	22.11	23.35	0.99
Crude ash	2.73	2.59	2.55	2.61	2.40	2.43	0.08
Amount of lactic acid bacteria (log CFU/g)							
LAB in diet	5.38^{b}	5.80^{a}	5.83^{a}	5.84^{a}	5.85^{a}	5.92^{a}	0.06
LAB in intestine	3.09^{a}	3.67^{b}	3.60^{ab}	3.42^{ab}	3.40^{ab}	3.32^{ab}	0.15
Digestibility (%)							
Dry matter	81.69	71.52	78.1	81.09	78.69	79.36	3.55
Crude protein	88.30^{ab}	88.85^{ab}	88.76^{ab}	91.06^{a}	86.85^{ab}	86.03^{b}	1.75

[*] Values with the same superscript in rows are not significantly different (P>0.05)

Discussion

In this study, there were no significant differences in growth performances, FCR and PER of fish fed with diets containing fermented soybean meal up to 50% of fish meal protein compared with control diet. However, feed efficiency decreased in tilapia fed FSM50 diet, due to decreased feed intake in this group. Feed intake was gradually reduced from 9.45g in control, to 7.91g in FSM50. This study showed that 40% SMFW could replace dietary fish meal without any significant negative impact on growth performance. This fish meal substitution level was higher than the level observed by both Shiau et al., (1990) and Olmez and Seyhan (2000) where the level of soybean meal in tilapia diets was 30%. This growth improvement may be attributed to the nutritional value and elimination of antinutritional factors when soybeans are fermented with whey. In the current study, soybean meal fermented with whey contained higher crude protein than unfermented soybean meal. There is little information on the use of fermented soybean meal in fish feeds. Fish meal in black sea bream diets could be replaced up to 20% by fermented soybean meal by *Candida utilis* (Zhou et al., 2011). Rainbow trout diets can contain up to 30% fermented soybean meal with *Bacillus spp*. and *Aspergillus spp* without any loss of rearing performance (Barnes et al., 2012). Fermented soybean meal using *Bacillus subtilis* with supplementation of methionine could replace up to 40% fish meal in juvenile black sea bream diets (Azarm and Lee 2012). Sesame seed meal fermented with lactic acid bacteria gave better growth performance than feeding raw sesame seed meal in rohu, *Labeo rohita* (Mukhopadhyay and Ray 1999). They attributed the growth improvement to the reduction of antinutrients such as tannin and phytic acid in raw sesame seed meal after fermentation process.

In the present study, HSI and VSI were not affected by feeding different levels of dietary soybean meal fermented by whey when compared with control group. No significant difference in HIS was found in juvenile red sea bream fed with fermented soybean meal (Kader et al., 2011). HSI and VSI were not affected by feeding different levels of fermented soybean meal in pompano diets (Lin et al., (2012). HSI was not affected by dietary fermented soybean meal level, however VSI was significantly increased with increasing amount of fermented soybean meal in diets (Azarm and Lee (2012). Body protein level also was not affected in fish fed dietary fermented soybean meal. Similar results were reported in previous studies (Azarm and Lee 2012; Kader et al., 2011; Lin et al., 2012).

Protein digestibility was not affected by different levels of dietary fermented soybean meal (P>0.05). Protein digestibility observed in this study are similar to those reported by Azarm et al., (2012) who showed that protein digestibility was not affected by dietary fermented soybean meal level in black sea bream. Protein digestibility significantly decreased with increasing dietary fermented soybean meal level in black sea bream (Zhou et al., 2011; Azarm and Lee, 2011). Protein digestibility was not statistically significant in yellowtail fed fermented soybean diets (Nguyen et al., (2013).

In conclusion, this study showed that SMFW could replace up to 40% of the fish meal protein without negative effects on the growth performance of tilapia.

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